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Citation for published version (APA):

de Vogel-van den Bosch, J., van den Berg, S. A., Bijland, S., Voshol, P. J., Havekes, L. M., Romijn, H. A., Hoeks, J., Van Beurden, D., Hesselink, M. K. C., Schrauwen, P., & van Dijk, K. W. (2011). High-fat diets rich in medium- versus long-chain fatty acids induce distinct patterns of tissue specific insulin resistance. *Journal of Nutritional Biochemistry*, 22(4), 366-371. <https://doi.org/10.1016/j.jnutbio.2010.03.004>

Document status and date:

Published: 01/04/2011

DOI:

[10.1016/j.jnutbio.2010.03.004](https://doi.org/10.1016/j.jnutbio.2010.03.004)

Document Version:

Publisher's PDF, also known as Version of record

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High-fat diets rich in medium- versus long-chain fatty acids induce distinct patterns of tissue specific insulin resistance[☆]

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Received 10 September 2009; received in revised form 25 February 2010; accepted 4 March 2010

Abstract

Excess dietary long-chain fatty acid (LCFA) intake results in ectopic lipid accumulation and insulin resistance. Since medium-chain fatty acids (MCFA) are preferentially oxidized over LCFA, we hypothesized that diets rich in MCFA result in a lower ectopic lipid accumulation and insulin resistance compared to diets rich in LCFA. Feeding mice high-fat (HF) (45% kcal fat) diets for 8 weeks rich in triacylglycerols composed of MCFA (HFMCT) or LCFA (HFLCT) revealed a lower body weight gain in the HFMCT-fed mice. Indirect calorimetry revealed higher fat oxidation on HFMCT compared to HFLCT ($0.011.0 \pm 0.0007$ vs. 0.0096 ± 0.0015 kcal/g body weight per hour, $P < .05$). In line with this, neutral lipid immunohistochemistry revealed significantly lower lipid storage in skeletal muscle (0.05 ± 0.08 vs. 0.30 ± 0.23 area%, $P < .05$) and in liver (0.9 ± 0.4 vs. 6.4 ± 0.8 area%, $P < .05$) after HFMCT vs. HFLCT, while ectopic fat storage in low fat (LF) was very low. Hyperinsulinemic euglycemic clamps revealed that the HFMCT and HFLCT resulted in severe whole body insulin resistance (glucose infusion rate: 53.1 ± 6.8 , 50.8 ± 15.3 vs. 124.6 ± 25.4 $\mu\text{mol min}^{-1} \text{kg}^{-1}$, $P < .001$ in HFMCT, HFLCT and LF-fed mice, respectively). However, under hyperinsulinemic conditions, HFMCT revealed a lower endogenous glucose output (22.6 ± 8.0 vs. 34.7 ± 8.5 $\mu\text{mol min}^{-1} \text{kg}^{-1}$, $P < .05$) and a lower peripheral glucose disappearance (75.7 ± 7.8 vs. 93.4 ± 12.4 $\mu\text{mol min}^{-1} \text{kg}^{-1}$, $P < .03$) compared to HFLCT-fed mice. In conclusion, both HF diets induced whole body insulin resistance compared to LF. However, the HFMCT gained less weight, had less ectopic lipid accumulation, while peripheral insulin resistance was more pronounced compared to HFLCT. This suggests that HF-diets rich in medium- versus long-chain triacylglycerols induce insulin resistance via distinct mechanisms.

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1. Introduction

Insulin resistance plays a key role in the pathogenesis of Type 2 diabetes mellitus and other metabolic disorders such as obesity and hypertension [1]. Insulin resistance is characterized by the reduced

ability of insulin to promote peripheral glucose disposal and suppress hepatic glucose output. It is currently believed that insulin resistance is predominantly a life style disorder with diet composition as one of the major determinants. Results from both epidemiological and animal studies suggest that the risk of developing insulin resistance is increased by consumption of a high-fat (HF) diet [2]. It is observed that lipids not only increase body weight but also accumulate outside the adipose depots in for example; muscle, liver and heart [3]. This ectopic fat accumulation has been suggested to be directly or indirectly responsible for an impaired insulin signaling and the development of insulin resistance [4]. Indeed, multiple studies have shown that mixtures of long chain fatty acids varying in the degree of saturation originating from palm oil, lard, olive oil, corn oil or safflower oil, negatively affect insulin sensitivity [5–8], paralleled by an increase in ectopic fat accumulation [5,6].

Medium chain triacylglycerols are fats comprised of fatty acids with a chain length of 6–12 carbons and found predominantly in

Abbreviations: EGP, endogenous glucose production; HFLCT, high fat long-chain triacylglycerol; HFMCT, high fat medium-chain triacylglycerol; LCFA, long-chain fatty acid; LF, low fat; MCFA, medium-chain fatty acid; Rd, glucose rate of disappearance.

[☆] Supported by TI food and Nutrition (Wageningen, The Netherlands). The study sponsor was not involved in the design or performance of the study.

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coconut oil and dairy products. Medium chain fatty acids (MCFA) differ in several aspects from long chain fatty acids; (a) their shorter chain length renders them water-soluble in the intestinal lumen and in the cytoplasm of target cells, (b) they are absorbed predominantly via the portal vein into the liver bypassing the lymphatic system [9] and (c) they are rapidly beta-oxidized [10] due to their independence of mitochondrial carnitine palmitoyl transferase 1, which is a fatty acid transporter and one of the rate limiting steps for mitochondrial beta oxidation.

Several studies have confirmed an increased fat oxidation and whole body energy expenditure in humans after 7–28 days diet intervention with medium chain fatty acids [11,12]. Since MCFA are preferentially oxidized, we questioned whether a HF diet rich in medium chain fatty acids would lead to less accumulation of fat in the skeletal muscle and the liver and thereby not impair insulin sensitivity. Therefore, we here examined if long-term substitution of medium chain triacylglycerols by long chain triacylglycerols in an isocaloric diet with similar amounts of proteins, fat and carbohydrates increases whole body fat oxidation and prevents long chain HF diet induced and insulin resistance of skeletal muscle and liver.

2. Materials and methods

2.1. Animals, diets and housing

Male C57Bl/6J mice were obtained from Charles River Laboratories at an age of 8 weeks and acclimatized up to an age of 12 weeks at the Leiden University Medical Centre animal facility. Animals were housed individually in a controlled environment (23°C, 40–50% humidity) under a 12 h light-dark cycle (07:00–19:00). Food and tap water was available ad libitum during the entire experiment. After acclimatization mice were randomized and kept on a low-fat (LF) diet [13,14] (RM3, Special Diet Services, Witham, Essex, UK) (Table 1), or a HF diet either rich in long-chain triacylglycerol (HFLCT) or medium-chain triacylglycerols (HFMCT) (45% energy in the form of fat, LCT; 4031.17, MCT; 4031.16, Hope Farms, Woerden, The Netherlands) (Table 1). The fatty acid composition of the diets is shown in Table 2. All animal experiments were approved by the Animal Ethic Committee from the Leiden University Medical Center in accordance with the principles and guidelines established by the European Convention for the Protection of Laboratory Animals.

2.2. Indirect calorimetry

Groups of eight mice per HF diet were subjected to individual indirect calorimetric measurements (Comprehensive Laboratory Animal Monitoring System, Columbus Instruments, Columbus Ohio, US) for a period of 4 consecutive days after 1 and 8 weeks of diet intervention [36,37]. A period of 24 h was included at the start of the experiment to allow acclimatization of the animals to the cages. This period was not used in the calculation and interpretation of the results. Oxygen consumption (VO_2) and carbon dioxide production rates (VCO_2) were measured at intervals of 7 minutes. Fat and carbohydrate oxidation rates were calculated from VO_2 and VCO_2 using the following formulas:

$$\text{Carbohydrate oxidation (kcal/h)} = ((4.585 \cdot \text{VO}_2) - (3.226 \cdot \text{VCO}_2)) \cdot 4 / 1000$$

$$\text{Fat oxidation (kcal/h)} = ((1.695 \cdot \text{VO}_2) - (1.701 \cdot \text{VCO}_2)) \cdot 9 / 1000$$

Total energy expenditure was calculated from the sum of carbohydrate and fat oxidation. Measured parameters also included real time food intake and activity, which was monitored as two-dimensional infrared beam breaks.

Table 1
Macronutrient composition of the diets

Ingredients	LF	HFLCT	HFMCT
	g/kg		
Protein	212	211	211
Carbohydrate	492	383	383
Fat	53	250	250
Fiber	46	61	61
	MJ/kg		
Energy	15.1	19.7	19.7

Table 2
Percentages of fatty acids in the diets

Percentage fatty acids of total lipids	LF	HFLCT	HFMCT
	%		
C8:0	–	–	37
C10:0	–	–	55
C14:0	1	1	–
C16:0	16	75	1
C18:0	3	6	–
C18:1	19	13	2
C18:2	48	4	4
C18:3	5	1	1
Other	8	–	–

All fatty acids are a percentage of total composition.

2.3. Net caloric intake

Feces were also collected during the stay in the metabolic cages in week 8. Feces were lyophilized and analyzed for energy content using adiabatic bomb calorimetry (Ikkalorimeter system C4000 Heitersheim, Germany). Net caloric intake by the animal was calculated by subtraction of the energy content of the feces from the gross dietary energy intake.

2.4. Histological analysis of intramyocellular and intrahepatic lipids

Cryosections (5 μm) from the liver and the midbelly region of the gastrocnemius muscle were stained for neutral lipids with Oil red O [15] and quantified as previously described [16].

2.5. Hyperinsulinemic euglycemic clamp

Hyperinsulinemic euglycemic clamps were performed as described before [17–21], with minor modifications. Clamp experiments were performed after an overnight fast and under anesthesia by intraperitoneal injection with a combination of Acepromazin (0.5 mg/kg, Sanofi Santé Nutrition Animale, Libourne Cedex, France), Midazolam (0.25 mg/kg, Roche, Mijdrecht, The Netherlands) and Fentanyl (0.025 mg/kg, Janssen-Cilag, Tilburg, The Netherlands).

A continuous infusion of D-[1- ^{14}C]glucose at a rate of 0.8 $\mu\text{Ci/h}$ (specific activity, 9.6 GBq/mmol; Amersham, Little Chalfont, UK) in the tail vein was started and blood samples were taken to determine basal glucose kinetics. After 60 min, the mice received a bolus of insulin (4.5 mU, Actrapid; Novo Nordisk, Chartres, France) to initiate the hyperinsulinaemic state followed by constant infusion of insulin (3.5 mU/kg per minute). Infusion of D-[1- ^{14}C]glucose was continued at a rate of 0.8 $\mu\text{Ci/h}$ and a variable infusion of D-glucose (12.5% w/v in phosphate-buffered saline) was used to maintain blood glucose levels at basal levels (euglycemia). Blood glucose was monitored via tail bleeding with a hand glucose meter (AccuCheck, Roche Diagnostics, Metronic Medical Systems, Vianen, The Netherlands) every 10 min to adjust the glucose infusion rate. After reaching steady state, blood samples were taken at 10-min intervals during 30 min to determine levels of D-[1- ^{14}C]glucose. An average clamp experiment took approximately 2.5 h, and anesthesia as well as body temperature was maintained throughout the procedure. Immediately after the last blood sample the mice were killed by cervical dislocation and hindlimb skeletal muscles and liver were dissected and frozen in liquid nitrogen-cooled isopentane and stored at -80°C until further analyses.

2.6. Analysis of clamp samples

To measure plasma D-[1- ^{14}C]glucose activity, trichloroacetic acid (final concentration 2%) was added to 7.5 μl plasma to precipitate proteins using centrifugation. The supernatant was dehydrated and resuspended in milliQ. The samples were counted using scintillation counting (Packard Instruments, Dowers Grove, IL, USA).

2.7. Calculations

The glucose turnover rate ($\mu\text{mol min}^{-1} \text{kg}^{-1}$) was calculated during the basal period and under steady-state clamp conditions as the rate of tracer infusion (dpm/min) divided by the plasma specific activity of D-[1- ^{14}C]glucose (dpm/ μmol). This ratio was corrected for total body mass. The hyperinsulinemic endogenous glucose production was calculated as the difference between the tracer-derived rate of glucose appearance and the glucose infusion rate.

2.8. Statistics

All data derived from the experiments were analyzed using the SPSS 15.0 package. Indirect calorimetry and net caloric intake was tested using 2-sided unpaired t-tests for normally distributed data. When comparing three groups analysis of variance (ANOVA) was used to calculate for significant differences between groups and

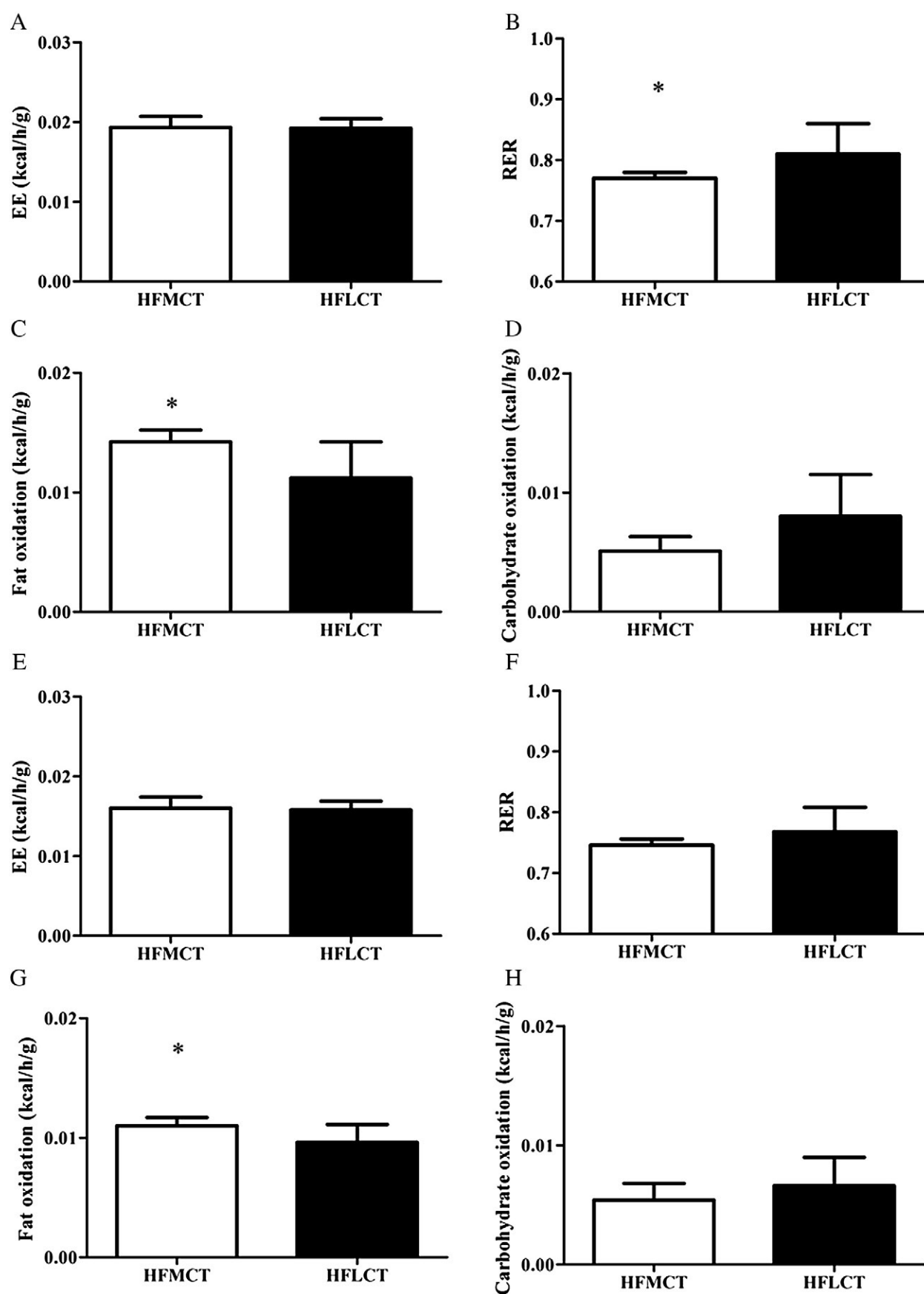


Fig. 1. Respiratory exchange rate, substrate oxidation rates and total energy expenditure after 1 (A–D) or 8 weeks (E–H) on a HFMCT or HFLCT diet. RER (A and E), energy expenditure (EE) (B and F) fat (C and G) and carbohydrate oxidation rates (D and H) are the mean values of the diurnal and nocturnal periods of the day (mean \pm S.D., $n=8$; * $P<0.05$ compared to the respective diet in the same week).

additional LSD post hoc tests to define the significance between groups. If ANOVA assumptions were violated, the results were tested with non-parametric Kruskal-Wallis ANOVA and Mann-Whitney *U* post hoc tests. All results are expressed as means \pm S.D. Statistical significance threshold was set at $P < .05$.

3. Results

3.1. Body weight and net caloric intake

Adult 12-week old male C57Bl/6 mice were maintained on a low-fat (LF) diet or transferred to a HF diet, enriched in triacylglycerols composed of long-chain fatty acids (HFLCT) or MCFAs (HFMCT) for a period of 8 weeks. At the end of the study, all HF-fed mice gained weight compared to the start of the experiment. After 8 weeks of diet intervention, body weight gain of the HF-fed mice was significantly higher compared to the LF-fed mice ($P < .02$) (LF, 1.8 ± 1.5 g; HFLCT, 5.6 ± 1.4 g; HFMCT, 3.8 ± 1.9). In addition, HFMCT mice gained significantly less weight compared to the HFLCT fed mice ($P < .05$). Total caloric intake over 70 h, determined during the metabolic cage analysis, was significantly lower in the HFMCT compared to HFLCT-fed animals at Week 1 (HFMCT, 1.84 ± 0.24 kcal/g; HFLCT, 3.11 ± 0.49 kcal/g; $P < .01$) and at Week 8 (HFMCT, 2.13 ± 0.34 kcal/g; HFLCT, 2.80 ± 0.48 kcal/g; $P < .05$). Caloric content of the feces collected during the stay in the metabolic cage in week 8 was significantly lower in the HFMCT compared to the HFLCT-fed mice (HFMCT, 0.16 ± 0.03 kcal/g; HFLCT, 0.35 ± 0.09 kcal/g; $P < .01$), which represented $\sim 7.5\%$ and $\sim 12.3\%$ of total caloric intake. Despite the higher fecal caloric output of the HFLCT-fed animals, net caloric intake was still significantly higher in the HFLCT-fed animals compared to the HFMCT-fed mice (HFLCT, 2.5 ± 0.4 kcal/g; HFMCT, 2.0 ± 0.3 kcal/g; $P < .02$).

3.2. Indirect calorimetry

Indirect calorimetry measurements were applied to examine whole body substrate utilization of the HF-fed mice during 3 consecutive days in Weeks 1 and 8. Total energy expenditure was similar in both HF diets after one ($P = 1.0$) or 8 weeks ($P = .8$) of dietary intervention (Fig. 1A and E). However, mean 24-h respiratory exchange rate (RER, defined as VCO_2/VO_2) in HFMCT-fed animals was significantly lower compared to HFLCT-fed animals in week 1 ($P = .05$) (Fig. 1B). In week 8, 24 h RER values tended to be lower in HFLCT-fed mice ($P = .07$) (Fig. 1F). These results indicate that HFMCT-fed animals have a HF to carbohydrate oxidation ratio compared to HFLCT-fed animals. Indeed, when calculating the 24 h fat and carbohydrate oxidation levels, a higher 24 h fat oxidation rate was found in HFMCT-fed mice in both week 1 ($P = .03$) and week 8 ($P = .02$) (Fig. 1C and G).

Calculated 24 h carbohydrate oxidation rates were not significantly different between HFMCT and HFLCT-fed mice at week 1 ($P = .07$) or week 8 ($P = .4$) (Fig. 1D and H). These data indicate that MCT feeding leads to higher relative and absolute fat oxidation levels compared to LCT feeding.

3.3. Ectopic lipid accumulation

To determine whether HFMCT and HFLCT diets resulted in differential ectopic lipid accumulation Oil red O staining of skeletal muscle and liver was performed (Supplementary Figs. 1 and 2). These experiments showed that the mice fed the HF diet enriched for MCT had significantly lower levels of intramyocellular lipid content compared to the HFLCT ($P < .03$, Fig. 2A). Staining of liver sections revealed a similar response with a significantly lower level of lipids in the HFMCT group compared to the HFLCT group ($P < .002$, Fig. 2B). Intramyocellular and intrahepatic lipid content in the LF-fed mice was very low and just above detection limits (Fig. 2A and B).

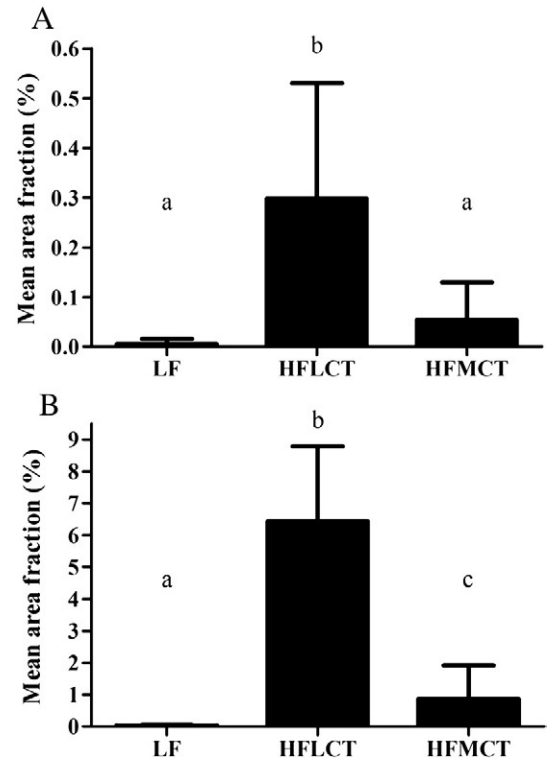


Fig. 2. Effects of an 8-week intervention with a LF, HFLCT or HFMCT diet on intramyocellular lipid content of the hind limb skeletal muscle (A) and the intrahepatic lipid content (B). Area fraction represents the percentage of the total muscle cell or liver area covered by lipid droplets. (mean \pm S.D., $n = 3-8$, bars with different letters are significantly different, $P < .05$).

3.4. Hyperinsulinemic-euglycemic clamp

To determine the effect of the HFMCT and HFLCT diets on whole-body glucose metabolism, hyperinsulinemic euglycemic clamps were performed in combination with continuous infusion of D-[^{14}C] glucose. From these experiments peripheral and liver insulin sensitivity can be calculated separately. Glucose infusion rates to maintain euglycemia during the clamp were not significantly different between both HF diets (Fig. 3), but both infusion rates were significantly lower compared to LF-fed mice ($P < .001$ for both HF groups vs. LF). Under basal conditions, glucose rate of disappearance (Rd) and endogenous glucose production (EGP) were not significantly different between groups (Fig. 4A and B).

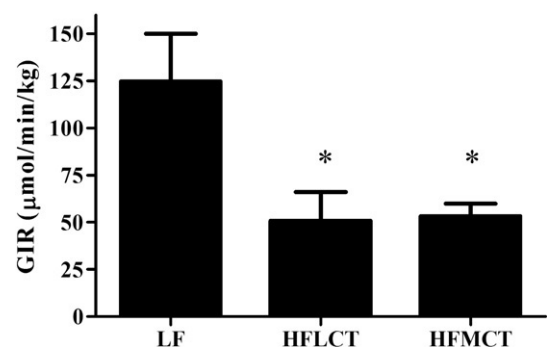


Fig. 3. Effects of an 8-week intervention with a LF, HFLCT or HFMCT diet on glucose infusion rate (GIR) during the hyperinsulinemic euglycemic clamp (mean \pm S.D., $n = 6-10$, * $P < .001$; LF vs HF).

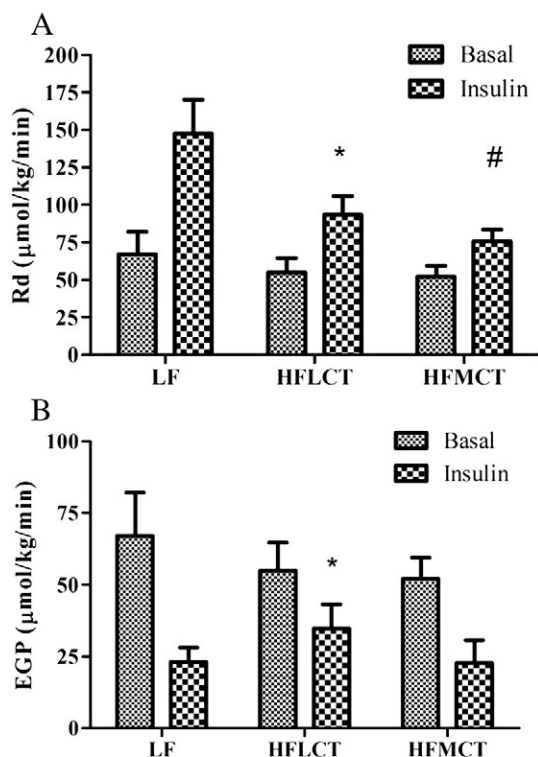


Fig. 4. Basal and insulin stimulated glucose disappearance rate (Rd) (A) and endogenous glucose production (EGP) (B) during the hyperinsulinemic euglycemic clamp (mean \pm S.D., $n=5-10$, * $P<.05$; HFLCT vs. LF and HFMCT; # $P<.005$, HFMCT vs. LF compared to the respective diet within the insulin stimulated state).

During the hyperinsulinemic state, Rd was significantly lower in the HFMCT-fed group compared to the HFLCT group ($P<.03$). Indicating impaired insulin sensitivity of the peripheral organs in the HFMCT compared to the HFLCT-fed mice. In addition, insulin stimulated Rd was significantly lower in both HF groups compared to the LF group ($P<.005$) (Fig. 4A).

In contrast, endogenous glucose production under hyperinsulinemic conditions was significantly lower – indicating improved hepatic insulin sensitivity – in HFMCT compared to HFLCT ($P<.05$). In addition, EGP in the HFLCT was significantly higher compared to LF ($P<.01$) (Fig. 4B).

These results indicate that both HF diets induced insulin resistance, although under hyperinsulinemic conditions peripheral insulin sensitivity was more reduced by the HFMCT diet, whereas liver insulin sensitivity was only affected by the HFLCT diet.

4. Discussion

In the present study, we showed that diets rich in medium chain triacylglycerols have much lower levels of lipids in skeletal muscle and liver when compared to diets rich in long-chain triacylglycerols. However, surprisingly, both HF diets reduced whole body insulin sensitivity to a similar degree. Therefore, medium chain triacylglycerol diets have no beneficial effect on the maintenance of whole body glucose metabolism. Since there is a clear difference in the development of tissue-specific insulin resistance, the mechanisms underlying the development of insulin resistance may, however, differ between long- and medium-chain diets.

Whole body insulin resistance is associated with increased dietary fat intake [2,22]. Therefore, it would be an ideal strategy to modulate whole body insulin sensitivity via a change in food

composition. In that context, MCT diets may be interesting. MCFAs are preferentially oxidized compared to long-chain fatty acids [10,23,24], first, due to their fast transport directly to the liver via the portal vein [9,25,26]; second, MCFAs are directed towards oxidation since fatty acyl synthetase responsible for triacylglycerol re-esterification is most sensitive for FA with a chain length of 14 or more [26] and third, because their transport into mitochondria is predominantly carnitine-independent.

Since a disturbed balance in fat intake vs. oxidation has been associated with the development of insulin resistance and Type 2 diabetes, foods that stimulate fat oxidation or inhibit food intake would be expected to have beneficial effects on insulin sensitivity. Here, we indeed show an acute and long-term adaptation with an increased 24-h fat oxidation after a diet rich in medium chain triacylglycerols when compared to LCT diets, as determined in whole body metabolic cages. Furthermore, mice fed the HFMCT diet ate less than the HFLCT-fed mice. This HFMCT-induced lower food intake might be explained by the level of liver fatty acid oxidation. Friedman et al. [27] have proposed that a decreased liver fatty acid oxidation, which reduces hepatic energy production, stimulates feeding behavior. In addition, it has been shown that increasing liver fatty acid oxidation, with for example feeding medium chain triglycerides, decreased food intake [28–30]. In addition, combining medium chain triacylglycerols in a breakfast of healthy human volunteers also decreased food intake by a post absorptive mechanism [31]. In the current study, we observed a higher fatty acid oxidation in the HFMCT compared to the HFLCT-fed mice. Although we do not know if the observed high fatty acid oxidation is specific for the liver, the peripheral tissues or both, we expect that due to the metabolic properties of MCTs, fatty acid metabolism in the liver is at least increased, which might explain the lower food intake in the HFMCT-fed mice.

The increased fat oxidation in combination with the lower net caloric intake of mice on the HFMCT diet compared to the HFLCT diet might explain the lower body weight gain. In contrast to previous reports [32,33], we did not observe any thermogenic effects of the HFMCT diets, as 24 h energy expenditure was comparable between HFLCT and HFMCT diets. More important, however, we confirmed our hypothesis that HF diets rich in medium chain fatty acids – in contrast to diets with a similar energy fat percentage in the form of long chain fatty acids – do not lead to the accumulation of lipids in skeletal muscle and liver. However, unexpectedly, both diets induced similar levels of whole body insulin resistance, when compared to LF diets. This illustrates that an accumulation of fat in liver and/or skeletal muscle is not a prerequisite for the development of whole-body insulin resistance.

Insulin-stimulated disappearance of glucose was lower in the HFMCT diet group when compared to the HFLCT diet group, whereas hepatic insulin sensitivity was reduced in the HFLCT but not in the HFMCT group. This indicates distinct regulation of the glucose metabolism in peripheral insulin sensitive tissues and the liver after both diets. In the HFLCT group, we speculate that whole body insulin sensitivity is impaired due to the ectopic accumulation of lipids that could interfere with insulin signaling in skeletal muscle and liver, as suggested previously [4]. The absence of hepatic insulin resistance in the HFMCT group fits with the concept that ectopic fat accumulation is associated with the development of insulin resistance, as the HFMCT diet, despite being high in fat content, did not result in the accumulation of fat in the liver. Most likely, HFMCT-induced peripheral insulin resistance is predominantly caused by a defect in glucose uptake in the skeletal muscles, given that skeletal muscle accounts for ~75–80% [34] of all insulin-mediated glucose uptake in the post prandial state. In that aspect, we can state that in the current model the induction of skeletal muscle insulin resistance by the HFMCT diet was not associated with accumulation of intramyocellular

lipids. The relationship between fatty acid metabolism and the development of Type 2 diabetes mellitus has in recent years been mainly explained by the accumulation of fatty acids in skeletal muscle that could interfere with insulin signaling. Randle suggested three decades ago [35] that competition of lipid substrates with glucose could be responsible for the development of insulin resistance. The results of the present study may fit with that concept. We observed a higher rate of fat oxidation in the HFMCT compared to the HFLCT-fed mice at all time points measured. We speculate that this high fatty acid oxidation rate in HFMCT-fed animals is due to a higher fat oxidation rate in the liver and skeletal muscles. Medium chain triacylglycerols are rapidly available and preferentially oxidized compared to LCT [10,23,24]. The reduced peripheral uptake of glucose might be due to the contribution of the myocellular MCFA-derived acetyl-CoA to the total pool of acetyl-CoA, which could create a negative feedback loop via citrate accumulation on pyruvate dehydrogenase. This sequentially blocks the glycolytic pathway and ultimately results in reduced glucose uptake and oxidation and thereby to a reduced insulin stimulated uptake of glucose [35]. However, further exploration of these substrate fluxes requires additional studies.

Taken together, a HF diet rich in medium chain triacylglycerols is preferentially oxidized over long chain triacylglycerols, which resulted in a lower accumulation of lipids in the skeletal muscle and the liver of the mice. Surprisingly, both HF diets induced whole body insulin resistance, although the HFMCT had a lower peripheral insulin sensitivity and normal hepatic insulin sensitivity compared to the HFLCT-fed mice. This suggests that these diets induce insulin resistance via distinct mechanisms.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jnutbio.2010.03.004.

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